



SCIENTIFIC PANEL ON FOOD ADDITIVES AND FLAVOURINGS (FAF)

MINUTES OF THE 24TH PLENARY MEETING – Open for Observers

**Held on 29-30 September 2021
Online meeting**

**14.00-18.00 on 29th September 2021 – Closed session
09.00-18.00 on 30th September 2021 – Open session**

(Agreed by written procedure on 20 October 2021)

Participants

■ Panel Members:

Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria José Frutos Fernandez, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy¹, Melania Manco, Wim Mennes², Peter Moldeus, Sabina Passamonti, Romina Shah, Dina (Ine) Waalkens-Berendsen, Detlef Wölfle, Matthew Wright and Maged Younes

■ Hearing Experts:

Riccardo Crebelli³ participated in agenda point 6.2

■ European Commission and/or Member States representatives:

DG SANTE (Health and Food Safety), E2 Food processing technologies and novel foods:
Catherine Evrevin and Jiri Sochor

■ EFSA:

FIP Unit: Claudia Roncancio Peña, Stefania Barmaz, Maria Carfi, Consuelo Civitella, Esraa Elewa, Marcello Laganaro, Federica Lodi, Carla Martino, Ana Maria Rincon, Antonio Rivas Cornejo, Camilla Smeraldi, Alkiviadis Stagkos-Georgiadis, Alexandra Tard, Giorgia Vianello and Riccardo Vríz

REPRO: Guilhem de Seze (HoD) for agenda item 9.2.1

¹ Apologies on 30th September 2021 PM

² Apologies on 30th September 2021 PM

³ Attended on 29th September 2021



■ Observers:

Ioannis Kartanos (PenTec), Michael Backes (Symrise AG), Zoltán Balázs (Leveret GmbH), Kirstie Canene-Adams (Mars Wrigley), Eric Chappuis (Cargill), Elena Cogalniceanu (EAS Strategies), Jan Demyttenaere (EFFA - European Flavour Association), Candace Doepker (ToxStrategies, Inc), Marta Duarte (IFF), Kristina Elvebakken (CP Kelco), Allison Franzen (ToxStrategies), Alisson Gebbie (Apeel Sciences), Stefanie Geiser (EAS Strategies), Maryse Herve (EU Specialty Food Ingredients), Frances Hunt (International Sweeteners Association), Zeynep Ilkbahar (Kerry), Christophe Lepretre (ICGA-Europe), Stefano Liparoto (Kerry Inc), Evangelia Mavromichali (SNE – Specialised Nutrition Europa), Petr Mensik (EPA, the European Association of Polyol Producers), Claudia Michel (BENEO GmbH), Eduardo Moraes (Givaudan), Francesca Ortolan (Cefic), Mari Reinik (Veterinary and Food Laboratory-Estonia), Caroline Rey (EFEMA), Rachel Serafin (AZELIS), Luca Terzi (FoodDrinkEurope) and Benjamin Voss (proFagus GmbH).

1. Welcome and apologies for absence | Closed session

The Chair welcomed the participants in the meeting. Apologies were received from Peter Fürst for the whole length of the meeting.

2. Adoption of agenda | Closed session

The agenda was adopted without changes.

3. Declarations of Interest of Scientific Panel members | Closed session

In accordance with EFSA's Policy on Independence⁴ and the Decision of the Executive Director on Competing Interest Management⁵, EFSA screened the Annual Declarations of Interest filled out by the Working Group members invited to the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process.

4. Agreement of the minutes of the 23rd Plenary meeting held on 22-24 June 2021, online meeting | Closed session

The minutes of the 24th Plenary meeting held on 22-24 June 2021 were agreed by written procedure on 30 June 2021⁶.

5. Report on written procedures since the 23rd Plenary meeting | Closed session

No scientific outputs were adopted by written procedure since the last plenary meeting.

⁴ http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf

⁵ http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf

⁶ <https://www.efsa.europa.eu/en/events/event/23rd-plenary-meeting-faf-panel>



6. Scientific topic(s) for discussion

FLAVOURINGS

6.1. Scientific opinion on 2-Hydroxy-4-Methoxybenzaldehyde [FLA-20-01] (EFSA-Q-2020-00573) | Closed session

The draft opinion on the application for authorisation of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] to be used as a new flavouring substance in and on food according to Regulations (EC) No 1331/2008 and (EC) No 1334/2008 was presented for the first time to the members of the Panel together with the main points for discussion.

The Panel discussed the different parts of the assessment and unanimously adopted the opinion, subject to incorporation of changes as suggested during the meeting.

The full opinion will be available on the Authority's webpage.

FOOD ADDITIVES

6.2. Progress update on the re-evaluation of sweeteners under Reg. (EC) No 257/2010: | Open session

- **Preliminary assessment of genotoxicity of: Mannitols (E 421 i, ii) EFSA-Q-2011-00646; EFSA-Q-2011-00647 and Sorbitols (E 420 i, ii) EFSA-Q-2011-00644; EFSA-Q-2011-00645**

Further to the discussions held at the previous Plenary meetings in May and June 2021^{7, 8}, the hearing expert and member of the FAF WG on Sweeteners, Riccardo Crebelli, joined the current plenary meeting for this agenda point to address questions from the Panel concerning the preliminary assessment of the genotoxicity data available for the re-evaluation of the two sweeteners, sorbitols (E 420) and mannitols (E 421).

As for the other substances presented at the previous plenary meetings, the aim of this preliminary assessment conducted by the WG Sweeteners was to establish whether the available data for the two remaining sweeteners included in the re-evaluation programme would be considered adequate with respect to the current standards (OECD test guidelines, EFSA SC, 2011⁹ and EFSA SC, 2017¹⁰) or whether the need for additional information considered relevant for the genotoxicity assessment had been identified in order to progress with the overall safety assessment.

- The Panel acknowledged that at this stage, based on the preliminary assessment of the available genotoxicity data for the two food additives, the need for additional data to be generated for sorbitols (E 420) or mannitols (E 421) was not identified.

⁷ <https://www.efsa.europa.eu/sites/default/files/2021-06/22nd-plenary-meeting-faf-panel-minutes.pdf>

⁸ <https://www.efsa.europa.eu/sites/default/files/2021-07/23rd-plenary-meeting-faf-panel-minutes.pdf>

⁹ EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011; 9(9):2379, 69 pp. <https://www.efsa.europa.eu/en/efsajournal/pub/2379>

¹⁰ EFSA Scientific Committee, Hardy, A, Benford, D, Halldorsson, T, Jeger, M, Knutsen, HK, More, S, Naegeli, H, Noteborn, H, Ockleford, C, Ricci, A, Rychen, G, Silano, V, Solecki, R, Turck, D, Younes, M, Aquilina, G, Crebelli, R, Gürtler, R, Hirsch-Ernst, KI, Mosesso, P, Nielsen, E, van Benthem, J, Carfi, M, Georgiadis, N, Maurici, D, Parra Morte, J and Schlatter, J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. <https://doi.org/10.2903/j.efsa.2017.5113>



- **Feedback on ongoing call for data on genotoxicity on: Acesulfame K (E 950) [EFSA-Q-2011-00721](#); Cyclamates (E 952 i, ii,iii) [EFSA-Q-2011-00733](#); [EFSA-Q-2011-00734](#); [EFSA-Q-2011-00735](#); Isomalt (E 953) [EFSA-Q-2011-00723](#); Sucralose (E 955); [EFSA-Q-2011-00724](#); Neohesperidine DC (E 959) [EFSA-Q-2011-00726](#); Neotame (E 961); [EFSA-Q-2011-00740](#); Salt of aspartame-acesulfame (E 962) [EFSA-Q-2011-00727](#); Lactitol (E 966) [EFSA-Q-2011-00728](#); Xylitol (E 967) [EFSA-Q-2011-00729](#);**

As an outcome of the previous discussions held at the plenary meetings in May and June 2021, a call for data aimed at gathering relevant information for the genotoxicity assessment of the substances above was published on the EFSA website on 30 June 2021¹¹.

A timeline of 6 months was indicated in the call for data for the submission of the requested information, in line with the period indicated in the EFSA document on 'Indicative timelines for submitting additional or supplementary information to EFSA during the risk assessment process of regulated products (update 2021)'.

The indicated timeline was considered sufficient by the experts for conducting the tests and reporting the data requested in the first instance (bacterial reverse mutation assay; *in vitro* micronucleus assay; FISH/CREST analysis of the *in vitro* micronucleus assay; *in vivo* Comet assay). Interested parties were asked to express their intention to submit the requested data by the 30th July 2021.

At the current meeting, the Panel was presented with an update on the responses received to the expression of interest to the call. EFSA explained that registration of interest in submitting the requested data was received for all the substances included in the call for data. However, almost all the registered interested parties that are committed to conduct the requested studies have also requested an extension of the deadline for submission of the data.

It was acknowledged that no specific technical issues were flagged to the attention of EFSA by any of the interested parties, and that the additional time requested was mainly needed for the sourcing of facilities that could conduct the tests.

On the other hand, EFSA and the Panel reiterated the importance of receiving the information requested to make progress with the assessment of the genotoxicity endpoint for the substances. While the assessment is advancing in parallel on all the other aspects of technical data, hazard identification and characterisation and of dietary exposure, availability of a complete dataset that would allow conclusions with respect to the potential genotoxicity of the substances, is pivotal for reaching final conclusions on the overall safety assessment.

All considered, the Panel agreed to the possibility of extending the deadline for submission of the data, proportionately to the initial timeline indicated for the generation of the new data (i.e. for an additional period of 3 months). The new deadline of 31 March 2022 will be applicable to all the substances included in the call, that will be updated accordingly and republished.

- **Preliminary assessment of technical data on Saccharin Na, Ca, K (E 954 i, ii, iii, iv) [EFSA-Q-2011-00736](#); [EFSA-Q-2011-00737](#); [EFSA-Q-2011-](#)**

¹¹ <https://www.efsa.europa.eu/en/call/call-data-genotoxicity-data-sweeteners>



00738; EFSA-Q-2011-00739; and Sucralose (E 955) EFSA-Q-2011-00724

The Chair gave a brief update on the preliminary assessment of the technical data (identity of the food additives, analytical data supporting the specifications, manufacturing process, etc) available for saccharins (E 954 i-iv) and sucralose (E 955). The Panel was informed that the preliminary assessment of the data has identified the need for additional information to be sought on these two food additives in order to complete this part of the safety assessment.

The Chair opened the floor to questions from the observers on this agenda item.

The following questions were posed by one of the observers attending the plenary:

- Can EFSA provide an explanation on the applicability of the new Transparency Regulation provisions to the re-evaluation of sweeteners, in particular with respect to the registration of studies?

EFSA clarified that the food additives re-evaluation undertaken under the frame of Regulation (EC) No 257/2010 is part of a mandate from the EC (M-2011-0160) sent to EFSA in 2011 and indicating the end of 2020 as the overall deadline for completion.

The new provisions introduced by the Transparency Regulation apply from 27 March 2021 in relation to re-evaluation procedures launched and follow-up steps taken from that date.

With respect to the submission of studies on the EFSA database on a voluntary basis, this is not foreseen according to the Practical Arrangements for the implementation of the Transparency Regulation.

6.3. Re-evaluation of thaumatin (E957) (EFSA-Q-2011-00725) | Open session

The draft opinion on the re-evaluation of thaumatin (E957) was presented to the members of the Panel together with the main points for discussion.

The Panel discussed the different parts of the assessment and unanimously adopted the opinion, subject to incorporation of changes as suggested during the meeting.

The full opinion will be available on the Authority's webpage.

6.4. Scientific opinion on the re-evaluation of Mono-and diglycerides of fatty acids (E 471) (EFSA-Q-2018-00100) | Open session

The draft opinion on the re-evaluation of on the re-evaluation of mono-and diglycerides of fatty acids (E 471) was presented to the members of the Panel together with the main points for discussion.

The Panel discussed the different parts of the assessment and unanimously adopted the opinion, subject to incorporation of changes as suggested during the meeting.

The full opinion will be available on the Authority's webpage.

The Chair opened the floor to the questions from the observers on this agenda item.

The following question was received during the registration phase:



- Are there any updated timelines for the publication of the EFSA opinions on additives in foods for very young infants under re-evaluation (tocopherols, silicon dioxide etc.) and an estimated timing for the publication of the additional EFSA calls for data for this group (such as E 472c, citrates etc.)?

Information on the status of the ongoing assessments may be found in the [minutes of the WG on the re-evaluation of food additives permitted in foods for infants below 16 weeks of age](#), regularly published on the EFSA website.

For what concerns the follow-up to the previous opinion on the re-evaluation of tocopherols (E 306, E 307, E 308 and E 309), it should be noted that EFSA has recently received a new mandate for a [scientific opinion on upper intake level of vitamin E](#), to be addressed by the the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) by March 2023. Since the assessment of the safety of the food additives to be carried out by the FAF Panel covers all population groups including infants below 16 weeks of age, it is considered relevant to wait for the NDA scientific opinion the upper intake level of Vitamin E before proceeding with the assessment of tocopherols (E 306, E 307, E 308 and E 309) as food additives.

With respect to the ongoing assessment of the follow-up data on silicon dioxide (E 551), the assessment of the data submitted by interested business operators on the characterisation of the silicon dioxide used as the food additive E551 (in particular on the particle size distribution) has been handed over to the [FAF WG on Specifications of Food additives](#).

The additional follow-up call for data on E 472c will be launched as soon as possible, pending on the workload of the FAF Panel.

Finally, for what concerns the other remaining food additives with original deadline for re-evaluation 31.12.2018 (such as citric acid and citrates) and for which the re-evaluation has not yet started, the timing of the launch of the call for data is not foreseen for the immediate future owing to the current workload of the Panel.

7. Introducing participants and presentation of the guidelines for Observers¹² | Open session

The Chair invited the members of the Panel and staff to introduce themselves to the Observers attending online.

The Scientific Panel coordinator presented the rules for observers to be followed during and after the open plenary meeting. Observers were given the possibility to send questions when submitting their registration and these questions would be answered in a dedicated session at the meeting. Observers were also informed that the Chair would grant opportunity for additional questions at the end of each discussion topic.

8. Other scientific topics for information and/or discussion | Open session

This agenda item was not discussed.

¹² <http://www.efsa.europa.eu/sites/default/files/observersguidelines.pdf>



9. Feedback from the Scientific Committee/Scientific Panels, EFSA, the European Commission | Closed & Open sessions

9.1. Scientific Committee and Scientific Panel(s) including their Working Groups | Open session

The Chair provided general feedback from the last meeting of the Scientific Committee which took place on 23-24 June 2021.

9.1.1. FAF Panel Working Groups (WG) | Open session

No additional issues were brought to the attention of the FAF Panel further to what is already recorded in the [minutes of the WG meetings](#), with the exception of the following two agenda items:

9.1.1.1. WG on Sulphur Dioxide-Sulphites (E220-228): feedback from the inaugural meeting

The Chair of the WG reported back from the inaugural meeting held on 19 August 2021. The mandate received from the European Commission, the conclusions of the previous re-evaluation opinion and the recommendations that are being followed up in the current assessment were presented to the Panel as well as information on the ongoing ECHA assessments on sulphur dioxide. An outline of the different areas of the assessment was also presented. The Panel noted that the part of the assessment related to the limits for toxic impurities in the food additives has been handed over to the FAF WG Specifications.

9.1.1.2. WG on Guidance Update on Flavourings: feedback from ad-hoc meeting with industry representatives on smoke flavourings

The Panel received feedback from EFSA on an [ad hoc meeting with industry representatives on smoke flavourings](#), organised on 30 June 2021 to discuss the new requirements for the assessment of smoke flavourings primary products as described by EFSA in the recently published documents for the preparation of applications on smoke flavouring primary products (EFSA FAF Panel, 2021¹³; EFSA, 2021¹⁴).

As an outcome of that meeting and of the follow-up questions received by EFSA after the meeting, the Question and Answer document (endorsed by the Panel at its 22nd plenary meeting in May 2021 and published as an annex to the plenary minutes¹⁵) deserves to be further elaborated to accommodate some of the points raised on the interpretation of the scientific guidance.

The Chair opened the floor to the questions from the observers on this agenda item. The following questions were received during the registration phase:

- According to Article 7 to Regulation (EC) No. 2065/2003, in order to obtain the inclusion of a smoke flavouring primary product (SFPP) in the Community list of authorised primary products, an application shall be submitted by sending it to the

¹³ EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2021. Scientific Guidance for the preparation of applications on smoke flavouring primary products. EFSA Journal 2021;19(3):6435, 40 pp. <https://doi.org/10.2903/j.efsa.2021.6435>

¹⁴ EFSA (European Food Safety Authority), 2021. Administrative guidance for the preparation of applications on smoke flavouring primary products. EFSA supporting publication 2021: 18(3):EN-6485. 32 pp. doi:10.2903/sp.efsa.2021.EN-6485

¹⁵ <https://www.efsa.europa.eu/sites/default/files/2021-06/22nd-plenary-meeting-faf-panel-minutes.pdf>



competent authority of a Member State. Member state will inform EFSA and make the application and any supplementary information supplied by the applicant available to EFSA. Article 12 of the same Regulation dictates that the same applies to applications for renewal of existing authorisations. Can you please clarify how the transparency regulation has changed the pathway for submission of an application for a SFPP?

- Given that we will only be able to submit a partial dossier / data package by the June 2022 deadline and additional data will accrue over time, what will EFSA do with the submitted data (in order to facilitate the timely re-authorisation of the materials / elongation of current approvals etc by EC) in the interim: start reviewing the data and issue an interim opinion or publish updated opinion (revisions) of the already existing opinions or just wait for the rest of the data and then review only complete packages?

In reply to the first question, EFSA clarified that according to Article 12 of Regulation (EC) No 2065/2003, applications for the renewal of the authorisations of smoke flavourings primary products should be submitted to the European Commission and not to the competent authority of a Member State. Concerning the impact of the Transparency Regulation *vis a vis* the submission pathway of an application for a smoke flavouring primary product, the requestor was referred to the relevant answer provided by EFSA in the context of the *ad-hoc* meeting held on 30 June, which is also reported in Annex A to these minutes (see EFSA's answer to Q.19). Regarding the second question, it was explained that this matter is still under discussion between EFSA and the Commission and that applicants will be informed in due course once a clear strategy will be agreed on how to tackle this issue.

The following question was eventually posed by one of the observers attending the plenary:

- The newly available web link to DietEx has not been included in the EFSA scientific guidance nor in the EFSA administrative guidance on smoke flavourings. Does EFSA plan to update these documents to include this link?

EFSA confirmed that both EFSA guidance e documents will be updated and republished to include the relevant web link to DietEx.

For transparency reasons and for the benefit of all interested stakeholders, the questions and answers discussed during and as a follow up to the *ad-hoc* meeting held on 30 June as well as those mentioned above discussed during the current plenary meeting are added to the Question and Answer document endorsed by the Panel at its 22nd plenary meeting in May 2021 and published in the [Annex A](#) below to the minutes.

9.2. EFSA including its Working Groups /Task Forces

9.2.1. Update on ART – Organigramme, RA workflow, DOI | Closed session

The Head of the REPRO Department, Guilhem de Seze, explained the reasons behind EFSA's new organisational structure. In order to implement the new obligations and new processes required by the Transparency Regulation (TR), the ART Programme designed and harmonised high-level Risk Assessment (RA) process divided in four main steps: Mandate & dossier intake; Preliminary activities to Risk Assessment; Risk Assessment and Output publication & dissemination. To best support the changes triggered by the TR, the next step was to redesign EFSA's internal structure: among other changes, EFSA will have one department focusing on RA production (Assess Department), and one focusing on services in support of RA production (Enable Department). The final organigramme, with all Heads of Unit, Teams and most Team



Leaders, was confirmed by the EFSA Management Team on 24th September, after which all staff (and experts) were informed.

Guilhem de Seze also gave an overview of the most recent DOI issues, explaining that while the technical Task Force is working on the issues reported, the automatic request for expert DOI submission was disabled from 18th September 2021. Experts will be asked to use the alternative workflow described in the EFSA Competing Interest Management rules. More information will follow via email.

9.3. European Commission

This agenda item was not discussed.

10. New Mandates | Open session

The Panel was informed of the following mandates received from the European Commission since the 23rd Plenary meeting held in June 2021:

10.1. Approval of liquid smoke for manufacture and marketing in the EU

This new mandate (M-2021-00615) relates to an application for the authorisation of a new smoke flavouring (EFSA-Q-2021-00398).

This request has been considered invalid by APDESK.

10.2. Request for a scientific opinion from the European Food Safety Authority on the proposed extension of use for polyglycerol polyricinoleate (E 476) in edible ices and emulsified sauces

This new mandate (M-2020-00609) from European Food Safety Authority covers the request for evaluation of an application on the proposed extension of use for polyglycerol polyricinoleate (E 476) in edible ices and emulsified sauces ([EFSA-Q-2021-00400](#)) and is under consideration by APDESK.

Pending confirmation of the validity of the application, the Panel noted that the present mandate may require to be handled jointly with the new mandate related to the specifications for the food additive polyglycerol polyricinoleate (E 476) (see agenda item 10.7)

10.3. Request for EFSA to perform a risk assessment and to provide a scientific opinion on the safety of buffered vinegar as a food additive

Since the last plenary meeting, this mandate (M-2021-00601) was considered valid by APDESK on 16/08/2021 and the scientific assessment is currently in progress ([EFSA-Q-2021-00349](#)). The WG Food Additives Applications is tasked with the drafting of the scientific opinion.

10.4. Request for EFSA to perform a risk assessment and to provide a scientific opinion on the safety in use of oligonucleotides as a food additive

Since the last plenary meeting, this mandate (M-2020-00605) was considered valid by APDESK on 27/08/2021 and the scientific assessment is currently in progress ([EFSA-Q-2020-00518](#)). The WG Food Additives Applications is tasked with the drafting of the scientific opinion.

10.5. Request for EFSA to perform a risk assessment and to provide a scientific opinion on the safety of the proposed extension of use for sucralose (E 955) in energy-reduced or without added sugar fine bakery wares



Since the last plenary meeting, this mandate (M-2021-00605) was considered valid by APDESK on 18/08/2021 and the scientific assessment is currently in progress ([EFSA-Q-2021-00375](#)). The WG on Sweeteners is tasked with the drafting of the scientific opinion that will be issued jointly with the re-evaluation.

10.6. Request for the European Food Safety Authority (EFSA) to perform a risk assessment and to provide a scientific opinion on the safety in use of polyphenol-rich extract as a food additive

Since the last plenary meeting, this mandate (M-2020-0082) was considered valid by APDESK on 27/09/2021 and the scientific assessment is currently in progress ([EFSA-Q-2020-00358](#)). The WG Food Additives Applications will be tasked with the drafting of the scientific opinion.

10.7. Other request under acceptance evaluation:

Since the last plenary meeting, the following mandates for following up on previous food additive re-evaluation opinions were received by EFSA:

- Specifications for the food additive polyglycerol esters of fatty acids (E 475)
- Specifications for the food additive polyglycerol polyricinoleate (E 476)
- Updated scientific opinion as regards the safety of calcium silicate (E 552), magnesium silicate (E 553a(i)), magnesium trisilicate (E 553a(ii)) and talc (E 553b) as food additives
- Updated scientific opinion as regards the safety of gold (E 175) as a food additive
- Scientific opinion as regards the safety of the food additive glycerol esters of wood rosins (E 445) and its specifications

As a first step the mandates above will be allocated to the WG Specifications for the assessment of the data related to the characterisation of the food additives.

11. Questions from and answers to Observers (in application of the guidelines for Observers) | Open session

The Chair opened the floor to any additional question from the observers attending the meeting.

No other general questions were raised by the observers, in addition to some clarifications on the points discussed during the open session of the plenary.

12. Any other business | Open session

None to be reported.



Annex A

Questions and answers on the [EFSA Scientific Guidance for the preparation of applications on smoke flavouring primary products](#)

Version 2 – discussed by Food Additives and Flavourings (FAF) Panel at its 24th Plenary meeting held on 28-30 September 2021. This version includes the main questions and answers discussed in the context of an *ad-hoc meeting* with industry representatives on smoke flavourings held by EFSA on 30 June 2011.

The answers provided to the questions listed below are without prejudice to the final decisions that EFSA may reach in future evaluations of smoke flavouring primary products.

Characterisation of smoke flavouring primary products

- 1. From an analytical point of view, it is still not very clear how the “tentatively identified fraction” and “tentatively identified molecules” will be treated and assessed. Most components of smoke flavours are not commercially available and therefore cannot be used for the comparison of chromatographic and mass spectral data. This will lead to numerous identified “tentatively” components.**

EFSA’s answer to Q.1:

As depicted in Figure C.1 of the [FAF Panel Scientific Guidance for the preparation of applications on smoke flavouring primary products](#), the genotoxicity assessment of smoke flavouring primary products is a two-step approach consisting of (i) conclusions on all identified components regarding their genotoxic potential and (ii) genotoxicity testing of the unidentified part of the Primary Product. The first step requires unequivocal chemical identifications of the individual components.

“Tentatively” identified components should be considered as part of the unidentified fraction of the primary product. As stated in the guidance document (section 1.2.4.4), any analytical information available to characterise the type and to estimate the proportions of chemical classes constituting the unidentified fraction should be presented. To this end, it is requested in the guidance document that the criteria underlying the tentative identifications should be clearly described (section 1.2.4.3.1). The more substantiated “tentative” identifications of components are, the more this information will assist in the assessment of the unidentified fraction.

- 2. The EFSA Guidance on smoke flavourings¹⁶ states: “if the detailed chemical analysis reveals changes in the chemical composition as a result of the modifications of the production process, this triggers the need for the submission of a new application.” Hence, there is a significant risk, if EFSA decides during evaluation that a new application is demanded.**

EFSA’s answer to Q.2:

¹⁶ EFSA FAF Panel, 2021. Scientific Guidance for the preparation of applications on smoke flavouring primary products. EFSA Journal 2021;19(3):6435, 40 pp. <https://doi.org/10.2903/j.efsa.2021.6435>



If the production process of an authorised primary product is modified, it is the responsibility of the applicant to assess the potential impact of these modifications on the chemical composition of the primary product and its compliance with existing specifications. As mentioned in the EFSA guidance on smoke flavourings, *"if the existing specifications of a primary product are not met or if the detailed chemical analysis reveals changes in the chemical composition as a result of the modifications of the production process, this triggers the need for the submission of a new application."*

Proposed uses and exposure assessment

3. The new 'EFSA exposure tool' mentioned in the EFSA guidance on smoke flavourings¹ in section 2.2 is not available. Hence it is unclear how exposure assessment will be conducted. In addition, the FAIM model will lead to a lot of uncertainty and anticipated overestimation of exposure.

Question discussed at the ad-hoc meeting on 30 June 2011

EFSA's answer to Q.3:

The FAIM tool is based on the food categories specified in Annex II, Part D of Regulation (EC) No 1333/2008¹⁷. This food categories nomenclature should also be referred to for the smoke flavourings. However, FAIM tool contains more detailed food categories that could be used with respect to those mentioned in the current legislation indicating the authorised uses of smoke flavourings (see Annex to Commission Implementing Regulation (EU) No 1321/2013¹⁸). Despite this, FAIM might still provide an overestimation of the dietary exposure as all foods within a food category are considered to contain the smoke flavouring primary product at the provided use level(s). This is mentioned in the guidance. The Dietary Exposure (DietEx) tool (i.e. the new EFSA exposure tool) has been released by EFSA on 1 September 2021 and is available at the following link: <https://www.efsa.europa.eu/en/science/tools-and-resources/dietex>. A user guide and a description of the main features of the tool are also available at: <https://www.efsa.europa.eu/sites/default/files/2021-08/dietex-features-instructions.pdf>

This tool is expected to complement results obtained with the FAIM tool, since it will allow the use of more specific food categories through the FoodEx2 classification system. Estimates of exposure will therefore be more accurate if the submitted use and use levels will be provided for detailed food categories. However, in this case, food categories will not be aligned with those specified in the food categories nomenclature used for smoke flavourings.

The exposure assessment will be conducted by EFSA as described in the guidance document, see section 2.2. Thus, applicants should provide estimates with both tools: FAIM (mandatory) and DietEx (optional). The initial margin of safety (MOS) for the smoke flavouring primary products should be calculated with the exposure estimates provided by FAIM. Exposure estimates from DietEx would potentially lead to less conservative estimates and may be used as a second step by the applicant. As part of the risk assessment, if needed, EFSA might further refine the exposure assessment with the

¹⁷ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354, 31.12.2008

¹⁸ Commission Implementing Regulation (EU) No 1321/2013 of 10 December 2013 establishing the Union list of authorised smoke flavouring primary products for use as such in or on foods and/or for the production of derived smoke flavourings OJ L 333, 12.12.2013, p. 54–67



aim to estimate the exposure as realistically as possible based on the provided data on use levels and on a more accurate selection of specific foods from the Comprehensive Database (e.g. through the use of facets) that are not available in the DietEx tool.

Genotoxicity

4. What is the recommended maximum dose to be used in *in vivo* genotoxicity studies of complex mixtures such as smoke flavouring primary products?

EFSA's answer to Q.4:

As mentioned by EFSA in the recently published technical report "[Outcome of the public consultation on the draft scientific guidance for the preparation of applications on smoke flavouring primary products](#)" (see Table 2, reply to question #21, pages 38-39), the range of concentrations or doses used in *in vivo* genotoxicity tests, from a maximum tolerated dose (MTD) to a dose producing little or no toxicity, should be established on the basis of the results of a preliminary range-finding study. This is in line with the recommendations given in OECD test guidelines.

Furthermore, in both OECD TG 474¹⁹ and OECD TG 489²⁰ it is reported: "*If the test chemical does not produce toxicity in a range-finding study or based on existing data, the highest dose for an administration period of 14 days or more should be 1000 mg/kg body weight/day, or for administration periods of less than 14 days, 2000 mg/kg/body weight/day. However, if the test chemical does cause toxicity, the MTD should be the highest dose administered and the dose levels used should preferably cover a range from the maximum to a dose producing little or no toxicity. For certain types of test chemicals (e.g. human pharmaceuticals) covered by specific requirements, these limits may vary.*"

In the case of smoke flavourings primary products, EFSA is of the view that if no toxicity is observed in an appropriately designed range-finding study, it would be appropriate to test higher doses than the above-mentioned maximum limits, in order to increase the dose of each of the individual components. If this resulted in toxicity, the corresponding dose would be considered sufficiently high.

However, in the absence of any toxicity, the highest dose to be applied is limited by the maximum volume that should be given to rodents. According to OECD TG 474 and 489, the maximum volume of liquid that can be administered by gavage at one time should not normally exceed 1 mL/100 g body weight except in the case of aqueous solutions where a maximum of 2 mL/100 g may be used.

5. The EFSA Guidance on clarification of some aspects related to genotoxicity assessments (EFSA SC, 2017)²¹ outlines that the demonstration of target tissue exposure in *in vivo* genotoxicity studies can be determined through the use of a weight-of-evidence-approach. Given that smoke flavourings are a complex chemical mixture, characterization of a single analyte within the mixture and establishment of toxicokinetic data would be challenging. In the absence of information to definitively confirm systemic exposure

¹⁹ OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 474: Mammalian erythrocyte micronucleus test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. <https://doi.org/10.1787/9789264264762-en>

²⁰ OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 489: In vivo mammalian alkaline comet assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. <https://doi.org/10.1787/9789264264885-en>

²¹ EFSA Scientific Committee, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. <https://doi.org/10.2903/j.efsa.2017.5113>



through detection of a single substance in the mixture from a specific blood/sample analysis can EFSA clarify what specific line of evidence outlined in the EFSA guidance from 2017 can be allowed to confirm systemic exposure for smoke flavours and provide certainty in a negative result?

Question raised at the ad-hoc meeting on 30 June 2011.

EFSA's answer to Q.5:

Concerning the demonstration of target tissue exposure in case of complex mixtures, the requestor is invited to refer to the considerations included in the Scientific Committee's statement on genotoxicity assessment of chemical mixture (EFSA SC, 2019²²) (page 8): *"If the in vivo testing of an in vitro positive mixture provides negative results, the relevance of the findings obtained in the in vivo follow-up tests will depend on the genetic effect assessed (i.e. gene mutations, structural or numerical chromosomal aberrations), the test protocol applied (route of exposure, tissues, etc.) and expert judgment on the reliability of the results obtained (including considerations of target tissue exposure). In some instances it can be anticipated that negative results in the follow-up tests can support, with sufficient confidence, a lack of concern about the in vivo genotoxicity of the mixture. For example, for a mixture that is directly clastogenic in vitro, a robust assessment in vivo could be performed by applying a mammalian alkaline comet assay (OECD (2016d) Test No. 489) to several tissues, including the site of first contact, to animals in which the mixture was administered orally. For other effects, such as induction of gene mutations and/or clastogenicity in vitro following metabolic activation, the assessment of systemic genotoxic effects (e.g. in the liver or bone marrow) may be limited by the fact that target tissue exposure cannot be demonstrated, as any toxic effect elicited in the target tissue by the mixture cannot be unequivocally attributed to the (in vitro) genotoxic component. In this scenario, the conclusion drawn would have a higher uncertainty. (...) So, for negative results in the in vivo follow-up study, the possible limitations of in vivo testing should be weighed in an uncertainty analysis before reaching a conclusion of no concern with respect to genotoxicity of complex mixtures that provided positive in vitro results."*

The following recommendations are also pointed out as may be relevant in this context:

- As recommended by the EFSA Scientific Committee ([EFSA Scientific Committee, 2011](#))²³, a combination of an *in vivo* micronucleus (OECD TG 474)³ and a comet assay (OECD TG 489)⁴, should be considered as a follow-up to a positive *in vitro* micronucleus assay.
- The range of doses to be applied in *in vivo* genotoxicity tests should reach the maximum tolerated dose (MTD) in line with the recommendations given in OECD test guidelines. If no toxicity is observed in an adequately designed range-finding study, it would be

²² EFSA Scientific Committee, 2019. Statement on the genotoxicity assessment of chemical mixtures. EFSA Journal 2019;17(1):5519, 11 pp. <https://doi.org/10.2903/j.efsa.2019.5519>

²³ EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. 69 pp. <https://doi.10.2903/j.efsa.2011.2379>



appropriate to test higher doses than the maximum limits mentioned in the OECD test guidelines, in order to increase the dose of each of the individual components of the mixture. The highest dose to be applied is limited by the maximum volume that should be given to rodents (1 mL/100 g body weight except in the case of aqueous solutions where a maximum of 2 mL/100 g may be used) (OECD TG 474³ and 489⁴).

6. In the case of Transgenic Rodent Mutation (TGR) assay (OECD TG 488)²⁴ conducted with a smoke flavouring primary product to follow up on potential concerns on mutagenicity, would the plasma analysis of a limited but representative “marker” components in the test item be regarded as sufficient evidence that the liver would be exposed, or at least sufficient to reduce the uncertainty related to the interpretation of the negative results of the TGR assay?

EFSA’s answer to Q.6:

Plasma analysis is not appropriate in this scenario as it is not possible to identify relevant marker components in smoke flavorings primary products, considering that a substantial part of the primary products may be unidentified and therefore the component(s) responsible for genotoxic effects *in vitro* cannot be unequivocally identified.

The range of doses to be applied in the TGR assay, as well as in other *in vivo* genotoxicity tests, should reach the maximum tolerated dose (MTD) in line with the recommendations given in OECD test guidelines. If no toxicity is observed in an adequately designed range-finding study (the parental strain of the transgenic mice could be used in case of TGR assay), it would be appropriate to test higher doses than the maximum limits mentioned in the OECD test guidelines, in order to increase the dose of each of the individual components of the mixture. The highest dose to be applied is limited by the maximum volume that should be given to rodents (1 mL/100 g body weight except in the case of aqueous solutions where a maximum of 2 mL/100 g may be used) (OECD TG 474³, 488⁸ and 489⁴).

If genotoxicity data are generated in the TGR assay on the first site of contact tissues where the local concentration of the test item is assumed to be highest (i.e. stomach and duodenum) and in the liver, there is no need to demonstrate liver exposure, provided that the treatment is performed at the MTD. The same considerations as described above also apply to the *in vivo* Comet assay.

Toxicity other than genotoxicity

7. The guidance provides a list of additional endpoints indicative for effects on the immune system to be assessed in the context of the 90-day oral toxicity study, to be conducted in line with OECD TG 408²⁵. Most of these parameters have never been used in regulatory rat studies under Good Laboratories Practices (GLP). Would it be possible to receive some explanation why all these parameters were added and receive some guidance and practical input on their assessment?

²⁴ OECD (2020), Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264203907-en>.

²⁵ OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD TG 408), in Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Publishing, Paris, <https://doi.org/10.1787/9789264304741-23-en>



EFSA's answer to Q.7:

The additional immunological parameters were added to the standard OECD TG 408 repeated dose 90-day oral toxicity study for renewal applications, to allow a full investigation of the potential effects on the immune system for the tested primary product. This option has been considered, following the comments from interested parties received during the public consultation of the draft guidance (see EFSA's responses to comments #5 and #27 in Table 2 of the "[Outcome of the public consultation on the draft scientific guidance for the preparation of applications on smoke flavouring primary products](#)"). Following these comments, EFSA's experts reconsidered the toxicological dataset originally requested for renewal applications, which included a full Extended One-Generation Reproductive Toxicity Study (EOGRTS), as currently required for applications for new smoke flavourings. Although, the EOGRTS remains the preferred option for renewals, an alternative option was considered appropriate by the EFSA's experts who developed the guidance, consisting of a 90-day toxicity study (OECD TG 408), with the additional parameters for the assessment of immunotoxicity, plus an OECD TG 414²⁶ prenatal developmental toxicity study in rats. This alternative has the advantage of (1) accommodating the time scale issue linked to Art 12 of Regulation (EC) No 2065/2003²⁷ and (2) allowing, at the same time, the identification of potential neurotoxic, endocrine, immunological (given the additional immunotoxicity parameters) and developmental effects.

The issue related to the need for developing and validating these endpoints according to GLP rules, e.g. the lack of historical control data, is acknowledged. However, while historical control data may be helpful for the interpretation of study results, the basis for the identification of treatment-related effects will in principle reside with the concurrent controls.

The methods for investigating these parameters have been in use for decades and their implementation in CRO laboratories should be feasible. As mentioned in the guidance document (section 3.3.3): "*some guidance for investigating these additional parameters may be found for example in 'Methods in Immunotoxicology' (Burlison et al., 1995), or in 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) or in the WHO/IPCS Guidance for immunotoxicity risk assessment for chemicals (WHO/IPCS, 2012).*"

As an additional guidance on where methodological input on the investigation of these endpoints can be found, please consider the following:

"At term (at sacrifice):

- *Histopathology (lymphatic organs(*)), including bone marrow cellularity;*
- *Weighing lymphoid organs(*)*

() Standard parameters in OECD TG 408."*

Regarding the investigation of bone marrow cellularity, the preferred method is to measure the number of cells in the suspensions prepared from bone marrow. Cell count data can be evaluated in conjunction with the histological examination of the bone marrow to judge effects of test articles on the hematopoietic system. However, examination of smears by an experienced pathologist may also provide sufficient information.

²⁶ OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 414: Prenatal Developmental Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264070820-en>

²⁷ Regulation (EC) No 2065/2003 of the European Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods. OJ L 309, 26.11.2003, p. 1–8.



"In blood:

- *Immunoglobulin isotypes*
- *Complement assays: total serum haemolytic activity or individual components*
- *C-reactive protein (CRP)*
- *Total and differential white blood cell count (*)"*

Kits are commercially available from different suppliers to investigate the above parameters.

Regarding the investigation of immunoglobulin isotypes, it is suggested to start with total IgG, IgM, IgA and IgE. If changes in total IgG are observed, it is recommended to evaluate IgG isotypes. For the complement assays, typically, C3 and C4 proteins should be measured. These complement markers should provide sufficient evidence of effects on the complement system.

"In spleen:"

In rodents, the analysis of leukocyte subpopulations and the functional tests as described below and in the EFSA guidance, are done typically using the spleen as a source of cells and not on peripheral blood. The issue with peripheral blood is that the amount of blood and cells that can be obtained from an animal will likely be insufficient. However, it may be considered acceptable to conduct some of these analyses, in blood rather than in spleen if the number of cells is enough.

- *Phenotypic analysis of spleen cells (CD4 and CD8 T cells, regulatory T cells, B cells, natural killer (NK) cells, macrophages)"*

Flow cytometry (FACS analysis) is a routine test, specific antibodies are available from different suppliers. In the case of rodents, typically this analysis is performed using splenocytes. See Chapter 12 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details.

- *Natural killer cell functional analysis"*

See Chapter 15 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details. Please consider that both the number and the functionality of the NK cells need to be investigated to get an indication of any potential alterations.

- *Phagocytic activity"*

This parameter may also be evaluated using splenocytes, see Chapter 17 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details. Kits are commercially available.

- *Mitogen stimulation assays for B and T cells"*

See Chapter 14 of 'Immunotoxicity testing. Methods and protocols' (DeWitt et al., 2018) for details. It is considered enough to measure cell proliferation after mitogen stimulation as this would provide an indication of the ability of B and T cells to undergo clonal expansion, which is central in the initial phase of the activation of acquired immunity. In case the applicant is interested in investigating the mechanisms of immunotoxicity, it may be advisable to analyse additional parameters, such as cytokine release or surface marker expression.

8. T-cell dependent antibody response (TDAR) assay is considered as a gold standard in the field of immunotoxicology to evaluate the humoral immune response to a T-cell dependent antigen. Kinetics of IgG and IgM against the T-cell dependent antigen can be used to evaluate any possible effects on the immunosystem. Is this test recommended by EFSA?

EFSA's answer to Q.8:



TDAR analysis is indeed a gold standard in immunotoxicology. In fact in the EFSA guidance on smoke flavourings it is stated that the preferred option to evaluate the safety of smoke flavouring primary products is to conduct an Extended One Generation Reproductive Toxicity study (EOGRTS), according to OECD TG 443²⁸, including a cohort specifically targeted to investigate the potential immunotoxicity of a test item, where TDAR assay is the prime element. However, in case an EOGRT study cannot be conducted due to timeline issues applicable to renewal applications, an alternative option has been recommended by EFSA which consists in a 90-study with the additional investigation of a wide range of immunological parameters, not foreseen in the EOGRT study protocol. This was dictated by an attempt to obtain as much information as possible on potential immunotoxicity from a subchronic oral toxicity study, without involving the use of additional animals.

- 9. In the EFSA guidance on smoke flavourings it is mentioned “A new 90-day oral toxicity study may be submitted according to the latest version of OECD TG 408⁵ (OECD, 2018a), including the assessment of neurotoxicity, since in the 90-day studies already available from previous submissions neurotoxicity was either not included or inadequately addressed”. It is assumed that this refers to the standard neurotoxicity parameters already included in the OECD TG 408 and no additions are needed. Is that correct?**

EFSA’s answer to Q.9:

Yes, this is correct. The standard neurotoxicity parameters included in OECD TG 408 repeated dose 90-day oral toxicity study are considered sufficient in this case, i.e. sensory reactivity to stimuli of different types such as auditory, visual and proprioceptive stimuli (functional observational battery (FOB)).

- 10. The guidance also mentions “This new oral 90-day toxicity study should also include a full assessment of parameters indicative of possible effects on the endocrine system as specified in Annex B of OECD TG 408”. Does this refer to the ‘required measures’ only or also the ‘optional measures’?**

EFSA’s answer to Q.10:

The parameters to be assessed for the detection of possible effects on the endocrine system are the ones specified in Annex B of OECD TG 408, including both the ‘required’ and the ‘optional measures’.

- 11. The legal deadline of June 2022 cannot be met by applicants when performing the Extended One-Generation Reproductive Toxicity Study (EOGRTS) or the alternative 90-day toxicity study with immunotoxicology parameters (see Options 2 and 3 in Appendix E of the EFSA guidance on smoke flavourings¹. Therefore, an alternative to the EOGRTS (i.e. the preferred data requirement in the EFSA guidance) has been designed. The details of this study design are available at this [link](#). It closer resembles the EOGRTS as it includes a reproduction and developmental phase which was not included in Option 3 in the EFSA guidance (i.e. the alternative 90-day study with immunotoxicology parameters). It also**

²⁸ OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264185371-en>



includes detailed assessment of endocrine disruption and immunotoxicology. With this study design, applicants will be able to meet the June 2022 deadline, there is no need to hire new personnel and it includes endpoints for which extensive experience and historical control data exist. Can you please indicate if applicants are allowed to follow this alternative path, although it is not the one recommended in the EFSA guidance?

Question discussed at the ad-hoc meeting on 30 June 2011.

EFSA's answer to Q.11:

During the meeting it was clarified that applicants may always deviate from EFSA's guidance documents and conduct alternative toxicological study designs to the ones recommended by EFSA, provided that they can justify their strategy and that the submitted alternative data could address the safety endpoints as indicated in the guidance, allowing to conclude whether the smoke flavouring primary product is safe under the proposed conditions of use.

12. Smoke Flavouring primary products were previously regulated using a margin of safety (MOS) of 300 because of the absence of specific toxicology data. As that data is now being requested in the EFSA guidance on smoke flavourings¹, could EFSA explain how additional uncertainty factors due to different toxicological data (especially regarding immunotoxicological endpoints) will affect the required MOS?

Question discussed at the ad-hoc meeting on 30 June 2011.

EFSA's answer to Q.12:

In 2010 the EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) stated: "...the Panel considered that, normally, an extra uncertainty factor of 3-fold in addition to the default uncertainty factor of 100, should be sufficient to cover the limited duration and statistical power of the pivotal study. However, each safety assessment requires expert judgement and should consider the data package on a case-by case basis. Whether a specific margin of safety for a particular smoke flavouring is sufficient, is highly dependent on the situation (e.g. composition, variability and stability, quality of the toxicological data,) and default guidance cannot be given" (EFSA CEF Panel, 2010)²⁹.

Therefore, the assessment factor of 300 should not be interpreted as a fixed value. It can be anticipated that good quality data for renewal/new applications on smoke flavourings will significantly reduce the chance that a higher factor than 300 will need to be applied in the risk assessment. However, it cannot be anticipated that a smaller assessment factor will become applicable with the standard data requirements as given in EFSA guidance on smoke flavourings¹. Immunotoxicological data should be treated as any other data and MOS does not need to be adjusted. Case by case considerations will apply in the safety evaluation.

13. EFSA stated in its answer to Q.7 that the additional immunotoxicology endpoints in the alternative 90-day toxicity study with immunotoxicology parameters (Option 3 in Appendix E of the EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021) were added to allow a full investigation of the potential effects on the immune system. However, the OECD TG 443 doesn't perform this extensive immunotoxicology assessment. Additionally, the alternative study excludes part of the assessment that would be

²⁹ EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids, 2010. Statement on the Safety Evaluation of Smoke Flavourings Primary Products: Interpretation of the Margin of Safety. EFSA Journal 2010; 8(1):1325. 7 pp. <https://doi.10.2903/j.efsa.2009.1325>



generated in the OECD TG 443 (i.e. developmental immunotoxicology). We have concerns regarding the lack of historical control data (e.g. validated endpoints) for certain immunotoxicology endpoints and the resulting reliance of the interpretation on concurrent controls within unvalidated, non-GLP methods. While the endpoints requested by EFSA have been in academic use, they have not been implemented in standard regulatory toxicology submissions, and they are not available among queried CRO's that represent roughly 90% of the global GLP testing capacity. Alternatively, an ICH S8 Immunotoxicology study³⁰ could be considered by applicants although this does not address developmental immunotoxicology.

Question discussed at the ad-hoc meeting on 30 June 2011.

EFSA's answer to Q.13:

As already mentioned in EFSA's answer to Q.7, the issue related to the adherence to GLP rules and the consequent current lack of historical control data for the immunotoxicology endpoints is acknowledged. Even if it is true that currently the endpoints are not included in regulatory toxicology submissions, assays to measure these endpoints have been in use for decades in several institutions including in university settings. While historical control data may be helpful for the interpretation of study results, any effects observed in treated animals will be evaluated against control animals, which will be sufficient to establish an effect deviating from controls.

The alternative approach to follow the recommendations of the ICH S8⁹ for pharmaceuticals is not recommended, as it does not include functional tests and it may result in undetected adverse effects.

14. Could EFSA share the ethical justification for the use of animals in validating the additional endpoints in the OECD TG 408⁹ study design that is not destined to be the preferred study moving forward.

Question discussed at the ad-hoc meeting on 30 June 2011.

EFSA's answer to Q.14:

The assessment of immunotoxicity is not a very well covered aspect in the current standards for toxicity testing. Additional validation studies for the requested parameters would certainly contribute to reduce the uncertainty with respect to the immunotoxicity assessment. It may also potentially contribute to a formal inclusion of selected parameters in the OECD test guidelines. For the suggested additional endpoints, established protocols are available and the use of additional animals is not foreseen.

15. Additional immunotoxicological assessments have been recommended in the EFSA guidance on smoke flavourings¹ which will require additional time, resources and use of animals for validation and will make it unlikely to meet the current legal timeline of June 2022 for the registrants. To overcome this issue, could EFSA consider a weight of evidence approach based on the integration of targeted immunotoxicology endpoints in the OECD TG 408, such as splenocyte immunophenotyping analysis, in-vivo cytokine analysis, keyhole limpet hemocyanin (KLH) stimulated T-cell-dependent antibody response (TDAR) analysis? This targeted approach would reduce the overall assay validation requirements and would be in line with the 3Rs principles.

³⁰ <https://www.ema.europa.eu/en/ich-s8-immunotoxicity-studies-human-pharmaceuticals>



EFSA's answer to Q.15:

EFSA understands the timelines constraints affecting the generation of the data required for renewal applications on smoke flavouring primary products. It is acknowledged that this issue also applies to the alternative data requirement for renewals as described in the EFSA's guidance on smoke flavourings, i.e. 90-day oral toxicity study (OECD TG 408⁹) with additional assessment of immunotoxicity, owing to the validation burden on the testing laboratories of the immunological parameters in order to incorporate them into the OECD TG 408 study.

Nevertheless, EFSA would like to reinforce that the recommendations included in the EFSA's guidance with respect to the investigation of potential immunotoxicity of smoke flavouring primary products are still appropriate.

Despite the involved testing laboratories may not have an in-house experience with NK cell functional analysis or with phagocytic activity using spleen cells, NK cell activity has been required by the Environmental Protection Agency (EPA) in the context of pesticides registration. Hence, standardised protocols in rodents are available for NK cell activity as well as phagocytic activity.

Concerning the alternative weight of evidence approach for an initial screening of the potential for immunotoxicity, please consider the following:

- Considering the relevance of immunotoxicity for both communicable and non/communicable diseases, we should move from a weight of evidence approach to a more conservative approach evaluating different immune function parameters in the context of a 90-day study, making the best use of animals, in any case sacrificed for the study itself. While it is true that assay requested are not targeted, EFSA guidance aims to have a better understanding of the possible adverse effects on the immune system addressing parameters relative to innate and adaptive immunity in a routine 90-days study. The suggested parameters do not require additional animals. Therefore, what EFSA has proposed is not going against the 3Rs principle. On the contrary, the proposed TDAR analysis will require additional animals. While currently TDAR is considered the gold standard in immunotoxicology, it requires satellite groups of animals, which will be against the 3Rs principle and it was not included in EFSA guidance for this reason. EFSA would support any additional tests complementary to the ones suggested in the EFSA guidance as long as no additional animals are needed, e.g. *in vivo* cytokine analysis (e.g. effect on Th differentiation), additional splenocytes immunophenotyping.
- Concerning the splenocyte immunophenotyping analysis and the *in vivo* cytokine analysis, it may be difficult to interpret the outcome of these assays and to observe real dose responses. Nevertheless, if dose responses can be shown, these analyses may be helpful to better understand the mechanism behind.
- Please consider that applicants may always deviate from EFSA's guidance, provided they can justify their strategy and adequate conclusions could be reached.

16. The EFSA Guidance on smoke flavourings¹ recommends the grouping of chemicals for a component based analysis be consistent with the criteria outlined in the ECHA R6



guidelines (ECHA, 2008)³¹ and practical guidance (ECHA 2012)³². However, the recent draft guidance on the grouping of chemical mixtures are inconsistent with the references outlined in the smoke flavour guidance. The EFSA Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019)³³ is focused on the grouping of chemicals primarily through a biological mechanism or pathway with a focus on data-rich chemicals. Will smoke flavours have to apply the grouping criteria outlined in EFSA's new guidance?

Question raised at the ad-hoc meeting on 30 June 2011.

EFSA's answer to Q.16:

As indicated in the EFSA guidance on smoke flavourings (EFSA FAF Panel, 2021, see sections 3.1. and 3.2) the possibility of grouping chemicals is offered only with respect to the genotoxicity assessment of the individual identified components. In this case the guidance prescribes that the genotoxic potential of the chemically identified components in smoke flavourings primary products should be assessed individually, using all available data. Structure-activity relationship (SAR) information about the genotoxic potential of an identified component may be considered when no adequate information on genotoxicity from published or unpublished studies is available. If a structural similarity with respect to potential genotoxicity is identified amongst the individual components, the selection of a representative substance could be considered for genotoxicity testing and used as indicator substance for all structurally related components that it represents. The criteria outlined in the ECHA R6 guidelines (ECHA, 2008) and practical guidance (ECHA 2012) should be applied for SAR considerations, for grouping the individual components based on structural similarity with respect to their potential genotoxicity and for the selection of representative substances for testing. The choice of a representative substance among the structurally related components should be justified, e.g. expected to have the highest genotoxic potential based on for example DNA/protein reactivity. On the other hand, the EFSA guidance makes clear that for primary products an individual evaluation should be performed, since they are complex mixtures for which read-across to other primary products is not applicable. In this case toxicity testing of the whole mixture should be considered for the derivation of a reference value (see sections 3.1. and 3.3). According to the principles outlined in the EFSA Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019), considering that smoke flavourings primary products are complex mixtures that may contain a substantial portion of unidentified components, the testing of the whole mixture of components for toxicity has the advantage of not only including individual components but could also reflect potential interactive effects of multiple components. Conversely, the genotoxicity assessment requires a combination of component-based and a whole mixture approach, since genotoxicity of individual components may not be detected in a whole mixture approach, e.g. as a result of dilution.

³¹ ECHA (European Chemicals Agency), 2008. Guidance for the implementation of REACH, Chapter R.6: QSARs and grouping of chemicals. ECHA, Helsinki. 134 pp. Available online: <https://echa.europa.eu/documents/10162/>

³² ECHA (European Chemicals Agency), 2012. Practical Guide 6. How to report read-across and categories. Version 2.0, December 2012. Available online: https://echa.europa.eu/documents/6362380/7127661/pg_report_readacross_en.pdf/69860e5b-c669-4a0d-b868-72f5dba5b560

³³ EFSA Scientific Committee, 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):5634, 77 pp. <https://doi.org/10.2903/j.efsa.2019.5634>



Uncertainty

17. It is still unclear how EFSA will apply uncertainty and what will be the impact on the overall safety assessment of the smoke flavouring primary products.

EFSA's answer to Q.17:

This question concerns uncertainty in future assessments of individual smoke flavouring primary products. Standard uncertainties (as listed in Appendix G of the [EFSA guidance](#)) require no further assessment as they have been considered by the Panel when developing the guidance document. Non-standard uncertainties will be identified by EFSA when conducting the assessment, following the criteria described in Appendix G of the guidance. When non-standard uncertainties are present, the combined impact of the non-standard uncertainties will be assessed by EFSA based on the available evidence plus expert judgement, following the approach outlined in section 4.2 of the 2018 [EFSA Guidance on Uncertainty Analysis in Scientific Assessments](#), and will be considered in EFSA's overall assessment of whether the smoke flavouring primary product achieves the level of safety required by the EFSA guidance¹. As explained in section 4.3 of the guidance document, applicants can contribute in reducing the uncertainties in the assessment by providing comprehensive information on all aspects of the risk assessment and doing every effort to fulfil the requirements as laid down in the guidance, using state-of-the-art approaches.

Procedural aspects

18. Considering the additional studies that have been requested in the updated EFSA's guidance document, it will be impossible for applicants to meet the submission deadline applicable to renewal applications of smoke flavourings primary products, as foreseen by Regulation (EC) No 2065/2003. In view of this issue, would it be possible to extend the legal deadline of 30 June 2022?

Question discussed by industry at the ad-hoc meeting on 30 June 2011

EFSA's answer to Q.18:

The deadline to submit renewals applications is set by the legislation. During the ad-hoc meeting on industry representatives on smoke flavourings held on 30 June 2011, the Commission representative confirmed that the legal deadline foreseen in Article 12 of Regulation (EC) No 2065/2003 applies and that applications for the renewal of existing authorisations must be submitted at the latest 18 months before the expiry date of the existing authorisations, i.e. by 30 June 2022. The Commission representative also indicated that for those smoke flavouring primary products for which a renewal would be requested in due time and for which delays in the renewal procedure would occur for reasons beyond the control of the authorisation holder, the Commission services were looking into options to ensure that there are no disruptions on the market while the applications would be examined.



19. As part of Regulation (EU) No 2019/1381³⁴ (the “Transparency Regulation”), applicants must submit a list of all intended studies used within the renewals according to Article 32c(1). This must be done prior to commissioning such studies. Due to the current time constraints, applicants dealing with renewals of smoke flavourings need to notify and commission studies immediately raising concerns that study start dates will be impacted and delayed if they follow the notification process for renewals.

- **In this scenario can applicants simultaneously submit a list of intended studies (Article 32c(1)) and notify these studies under Article 32b to ensure promptness in study commissioning?**
- **The Transparency Regulation only applies to EU-based laboratories, but are UK-based, non-EU contract research laboratories (CROs) under the oversight of the Transparency Regulation and thus require co-notification)?**
- **Can you please clarify how the Transparency Regulation has changed the pathway for submission of an application for a smoke flavouring primary product?**

Question raised by industry at the ad-hoc meeting on 30 June 2011.

EFSA’s answer to Q.19:

The new provisions introduced by Regulation (EU) 2019/1381¹⁹ (the “Transparency Regulation”), amending Regulation (EC) No 178/2002³⁵ (the “General Food Law”), related to the pre-submission phase of an application (i.e. general pre-submission advice, list of intended studies for renewal pre-submission advice, notification of studies) were presented by EFSA (presentation available at this [link](#)). In particular, EFSA clarified that in the context of renewal applications, if a study has been commissioned or started before 27 March 2021, the provisions laid down in Articles 32c(1) and 32b(2) and (3) of the GFL do not apply even if on 27 March 2021 the study is still ongoing.

Detailed answers to the specific questions raised by industry as described above are available in the “Questions and answers on the EFSA Practical Arrangements”³⁶, see Q.22 (page 16), Q23 (pages 16-17), Q.32 (page 20), Q.33 (page 20-21) and Q.35 (page 21).

In addition to the notification of studies, please note that the Transparency Regulation introduced several provisions applicable smoke flavouring applications for renewal. The new measures are detailed in the documentation below:

- Practical arrangements on pre-submission phase and public consultations ([here](#))
- Practical arrangements concerning transparency and confidentiality ([here](#))
- Question and Answers on the EFSA practical arrangements ([here](#))

³⁴ Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC *OJ L 231, 6.9.2019, p. 1–28* <http://data.europa.eu/eli/reg/2019/1381/oj>

³⁵ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety *OJ L 31, 1.2.2002, p. 1–24* <http://data.europa.eu/eli/reg/2002/178/oj>

³⁶ <https://www.efsa.europa.eu/sites/default/files/2021-03/questions-and-answers-efsa-practical-arrangements.pdf>



- EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products (update 2021) ([here](#)).

Furthermore, in order to support applicants better, in 2021 EFSA delivered an extensive training program available at the link [here](#). In particular we would like to point your attention to: Notification of Studies and Pre-submission advice (16/02 and 25/03), e-submission food chain platform (ESFC) (20/01, 05/02, 25/02, 09/03), Confidentiality (14/04, 18/05).

Dedicated webinars to explain the application procedure have been scheduled as of June 2021. In particular, we invite you to watch the one for food enzymes, food flavourings and food additives (04/06) and the one for feed additives (08/07) as it covers the aspects related to the submission of an application for renewal.

Lastly, we are pleased to inform you that EFSA has recently launched a new LinkedIn group to support regulated products applicants in understanding the application processes and tools. To join our community, please register at the link [here](#).

All links to access to the new EFSA portals are available in the Toolkit webpage [here](#).

In case applicants still need further support on the rules applicable to and the content of a specific application, then they can submit a request for general pre-submission advice, as defined in Art. 32a(1) of the GFL as amended by the Transparency Regulation, after registering to [EFSA.Connect](#).

Note

It is reminded that EFSA has implemented several initiatives to support applicants in understanding the evaluation process of applications for regulated products and to engage with them during all phases of the life-cycle of applications, including pre-submission activities such as general pre-submission advice. For the different possibilities of interaction with EFSA in the different phases of the application life-cycle, please consult [EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products](#).